	no integration of DNA*. Can infect stationary cells.	cells. Factors controlling tropism poorly understood. Generation of replication competent virus.		dystrophy, cancer.
Adeno-Associated Virus	Integration at specific sites*.	Requires replicating adenovirus to grow. No helper cell line. Specific integration probably does not occur in absence of viral genes. Very limited insert size.	Moderate	Similar to adenovirus.
Herpesvirus	High titers. Neurotropic*.	Complex construction. No packaging cell lines.	Slight	Neurologic disorders.
Poxviruses	High titers. Large insert size. High expression.	Highly immunogenic. Similar to adenovirus and herpesvirus.	Moderate	Localized, transient in vivo treatment.
Naked DNA	Easy to prepare in quantity. High level of safety*. Virtually unlimited size. No extraneous genes or proteins to induce immune response. Lack of integration*.	Very inefficient entry, uptake into nucleus. No mechanism for persistence or stability.	Moderate	Topical applications, mechanical and accessible (skin, vascular, pulmonary, endothelial cells).
Facilitated DNA (e.g., liposomes)	Same as DNA. More efficient uptake than DNA. Protected from in vivo Targetable to specific cell types*.	Targeting not yet achieved. No mechanism for persistence or stability. Inefficient entry.	Slight	As for naked DNA.

* Denotes theoretical advantage or concern, but one that has not yet been adequately tested.

Table 2. Delivery Vehicle of Clinical Gene Transfer Studies

System	# of Protocols	Percentage
Retrovirus vectors	76	71.7
Adenovirus	15	14.2
Adeno-associated viruses	1	0.9
Cationic liposome complex	12	11.3
Plasmid DNA	2	1.9

Table 3. Categories of Clinical Gene Transfer Protocols

Category	Disease/Disorder	# of Protocols	Percentage
Inherited Monogenic Disorders	**Total**	**20**	**18.9**
	ADA deficiency	1	0.9
	Alpha-1-antitrypsin	1	0.9
	Chronic granulomatous disease	1	0.9
	Cystic fibrosis	11	10.4
	Familial-hypercholesterolemia	1	0.9
	Fanconi anemia	1	0.9
	Gaucher disease	3	2.8
	Hunter syndrome	1	0.9
Infectious Diseases	**Total**	**8**	**7.5**
	Human inmunodeficiency virus-1	8	7.5
Acquired Disorders	**Total**	**2**	1.9
	Peripheral artery disease	1	0.9
	Rheumatoid arthritis	1	0.9
Cancer (byapproach)	**Total** Antisense	**51**	**49.1**
	Chemoprotection	2	1.9
	Immunotherapy/ex vivo	4	3.8
	Immunotherapy/in vivo	23	21.7
	Pro-drug/HSV-TK/ganciclovir	7	6.6
		11	10.4
	Tumor suppressor gene	4	3.8
Marking Protocols		**25**	**23.6**
All Studies		**106**	**100.0**

Data from Debra J. Wilson, Executive Secretary, Subcommittee on Data Management, Office of Recombinant DNA Activities, NIH

Appendix A

Panel to Assess the NIH Investment in Research on Gene TherapyPanel Members
Stuart H. Orkin, M.D. (Co-Chair)Haig H. Kazazian, Jr., M.D. Howard Hughes Medical Institute Department of Genetics Harvard Medical School University of Pennsylvania Division of Hematology-Oncology School of Medicine Children's Hospital Philadelphia, PA 19104-6145 Boston, MA 02115 **Arno G. Motulsky, M.D. (Co-Chair) Thomas J. Kelly, M.D., Ph.D.**Medicine-Medical Genetics Department of Molecular Biology University of Washington, RG-25 Johns Hopkins University Seattle, WA 98195-0001 School of Medicine Baltimore, MD 21205 **Richard Axel, M.D. Robert J. Lefkowitz, M.D.** Howard Hughes Medical Institute Howard Hughes Medical Institute Columbia University Duke University New York, NY 10032 Durham, NC 27710-0001 **David Botstein, Ph.D. Bernard Moss, M.D., Ph.D.** Department of Genetics National Institute of Allergy Stanford University and Infectious Diseases Stanford, CA 94305-5120 National Institutes of Health Bethesda, MD 20892 **John M. Coffin, Ph.D. Thomas A. Waldmann, M.D.** Department of Molecular Biology National Cancer Institute Tufts University School of Medicine National Institutes of Health Boston, MA 02111 Bethesda, MD 20892 **Pamela B. Davis, M.D., Ph.D. Huda Y. Zoghbi, M.D.** Pulmonary Division Department of Pediatrics Case Western Reserve University Baylor College of Medicine School of Medicine Houston, TX 77030-3411 Cleveland, OH 44106-2624 **Eric R. Fearon, M.D., Ph.D.** ***Executive Secretary*** Division of Molecular Medicine **Judith H.**

Greenberg, Ph.D. and Genetics National Institute of University of Michigan General Medical Sciences Medical Center National Institutes of Health Ann Arbor, MI 48109-0638 Bethesda, MD 20892**Uta Francke, M.D.** ***Program Assistant*** Department of Genetics/Beckman Center**Janice C. Ramsden** Howard Hughes Medical Institute Office of the Director Stanford University Medical Center National Institutes of Health Stanford, CA 94305-5428 Bethesda, MD 20892

Appendix B

REPORT OF THE FIRST MEETING, MAY 15-16, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as cochairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its first meeting on May 15-16, 1995, at the National Institutes of Health (NIH), Natcher Building, 9000 Rockville Pike, Bethesda, MD 20892. During the course of the twoday meeting, panel members heard from Dr. Harold Varmus, NIH Director, and more than 20 additional NIH representatives. Dr. Varmus delineated the panel's mandate, and other NIH staff members described current extramural and intramural programs supporting or otherwise affecting research on gene therapy.

Panel Mandate-Dr. Harold Varmus, NIH Director

Despite many challenges since the first gene transfer experiments were undertaken in microorganisms, biomedical researchers have made considerable progress toward realizing genebased therapies for human disease. Although clinical application of this emerging technology is still in an early phase of development, since 1988 the NIH Recombinant DNA Advisory Committee (RAC) has approved more than 100 protocols that involve tests of gene transfer or putative gene therapy procedures in clinical settings. Another panel, the Ad Hoc Review Committee of the RAC, which is chaired by Inder Verma of the Salk Institute, is examining how RAC functions in its role as reviewer of proposals to conduct clinical trials involving such gene transfers.

In the aggregate, NIH invests nearly $200 million annually in programs supporting and overseeing gene therapy research. Despite enthusiastic interest and early signs of safety and biological feasibility, however, evidence for therapeutic benefit to patients is meager. Moreover, opinions vary as to what gene delivery systems will prove effective over the long term, and there are unsettled questions as to which diseases are appropriate targets for gene therapy during this phase of its development.

The mandate for the Panel to Assess the NIH Investment in Research on Gene Therapy is to review broadly the gene therapy research enterprise, considering (i) current and proposed investments by NIH centers and institutes in gene therapy and related disciplines, (ii) developments affecting gene therapy in the wider community of academic, government, and industrial laboratories, and (iii) evaluation of the NIH investment in the context of other support for gene therapy research, particularly from the U.S. biotechnology industry and also from outside the United States.

From this comprehensive review, the panel is expected to devise a set of recommendations on NIHsponsored gene therapy research-not a rigid plan-to be presented at the meeting of the Advisory Committee to the Director, NIH, in December 1995. The recommendations are expected to help in NIH budget and program planning for FY 1997 (and, to a limited extent, FY 1996) by addressing specific questions, including the following:

- How should funds and efforts be distributed among areas such as gene delivery system

development, gene expression, biology of target cells, pathophysiology, and animal models of disease?
- What diseases and organ system targets should be emphasized during this period of gene therapy's development?
- What funding mechanisms will be most effective to meet specific program needs? What should be the roles of Requests for Applications (RFAs); centers; the NIH intramural program; pilot production facilities for developing and handling genes, vectors, and target cells; and training programs?
- How should NIH deal with policy issues such as patents and licenses, and what are the needs for public and professional education on the science and ethics of gene therapy?

The panel is also encouraged to make additional recommendations on how NIH might coordinate interdisciplinary gene therapy-related activities. For example, should NIH consider setting up a central coordinating office for such research? Moreover, the panel should also examine the impediments to progress in this field. In a broader context, panel members are reminded that the overall NIH budget is not likely to grow but is more likely to stay flat or be reduced in the near future. Hence, if increases in gene therapy research are deemed valuable and necessary, they will necessarily come at the expense of other programs.

NIH Staff Presentations

More than 20 NIH staff members presented information to the panel describing extramural and intramural programs that support or are otherwise relevant to the conduct of gene therapy research. These presentations ranged widely and included descriptions of major and more modest basic and clinical research programs being supported by several institutes and centers, information about grant and contract support mechanisms that may be applicable to future extramural gene therapy programs, available oncampus facilities and current research programs, plans to support a new vector and gene delivery development program, RAC's procedures for conducting reviews of clinical protocols and its experience developing a database for gene transfer clinical trials now under way, and current U.S. patent and licensing policies affecting research in this field.

The National Heart, Lung, and Blood Institute (NHLBI) ($53 million); the National Cancer Institute (NCI) ($10 million); the National Institute of Allergy and Infectious Diseases (NIAID) ($16 million); and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) support the largest efforts in gene therapy research, with seven other institutes sponsoring smaller programs. In addition, the National Center for Human Genome Research, in cooperation with researchers from several other institutes, is developing basic and clinical research projects strictly as part of its intramural program.

The NIH intramural program, from which the first several clinical protocols to be approved arose, continues to have a strong focus on gene therapy research. The wide variety of projects on the NIH campus to study disparate diseases, particularly rare disorders; specialized facilities, including stateoftheart human stem cell processing and transfer technology; an emphasis on highrisk, lab bench-to-bedside research at the clinical center; a concerted effort to reinvigorate the intramural program that features stringent staff reviews and a new tenure track system; and recently mandated incentives to encourage technology transfer from federal laboratories to the private sector are some of the reasons behind this focus. Recently, some 100 researchers in the intramural program formed acampuswide interest group.

A variety of funding mechanisms is available for supporting gene therapy efforts through theNIH extramural program. Researchers may submitinvestigatorinitiated grant applications, usually R01s, or prepare applications in response to RFAs, which invite investigators to submit proposals for projects in NIHspecified research areas. Typically, NIH commits funds forRFAs that it issues, and applications receive special reviews. Nonetheless, RFAs allow considerable latitude for researchers at different institutions to establish innovative arrangements and to set up collaborative networks.

In addition, there is a more formal grant mechanism for forming specialized multidisciplinary research centers at single institutions or among several institutions in a "Centers without Walls" program. Besides these grant mechanisms, the extramural program also can designate areas for competitive proposals to do contract research and development projects, usually with very specific targets. Beyond these standard funding measures, the NIH Director now has discretionary authority to transfer 1 percent ofNIH funds for a particular fiscal year into research areas of special interest or need.

Additional research resources supported by the extramural program of the National Center for Research Resources (NCRR) are part of a nationwide research infrastructure that already supports some gene therapy research activities and could be tailored or expanded to support additional efforts. For example, 14 of 75 general clinical research centers, most associated with U.S. medical schools, are conducting gene transfer trials. A biotechnology resource center now at Louisiana State University maintains an extensive, everexpanding database for human genemapping studies. There are seven regional primate research centers where gene therapy animal model studies can be conducted. As part of a new resource, three Institutes (NCI, NHLBI, and NIDDK) will begin supporting in mid1995 one to three national gene vector laboratories, whose establishment is based on a $3.5 million setaside for a joint RFA.

Another important element ofNIH's overall involvement in gene therapy research is the role it plays in overseeing policy matters such as the review of clinical protocols. As of May 1995, RAC has recommended approval for 105 human gene transfer protocols, including 77 involving some form of cancer, 19 involving various genetic disorders, and 8 on AIDS. Of this total, 25 aregenemarking experiments without any direct therapeutic potential. RAC is now streamlining its review procedures, and full responsibility for several categories of review now resides with FDA.

The NIH Office of Technology Transfer (OTT) serves under a congressional mandate to evaluate research and technology supported by the intramural program and to take appropriate steps to ensure that such intellectual property is further developed. Thus, OTT helps in identifying patentable inventions and filing applications, coordinating the development of cooperative research and development agreements (CRADAs) and material transfer agreements with researchers in industry or at universities, and arranging licensing agreements with industrial partners that seek to develop commercial products. NIH researchers, primarily from NCI and NHLBI, have filed 81 gene therapy-related patent applications (some of them diagnostic developments and others research tools). To date, NIH has completed 22 licenses covering gene therapy-related technologies.

Panel Deliberations

Panel members began to identify problems to address and their general approach for using the next two panel meetings. In general, the panel agreed to invite a total of 12-15 expert speakers to the two meetings, one to be held in Bethesda, Maryland, in July and the other in San Francisco, California, in August. Speakers will be asked to address a series of specific scientific issues affecting gene therapy research, including gene expression; stem cell biology; viral vector and other gene delivery systems; clinical disorders that are targets for gene therapy approaches, including cancer, AIDS, and inherited diseases; industry involvement; and patenting issues. Although an effort will be made to split the two meetings

thematically, with the first emphasizing basic science and the second emphasizing applied issues, other constraints from scheduling on relatively short notice may override that design.

The invited speakers, who may include leading exponents in this field and critics, will be asked to focus generically on an assigned topic, not merely to provide a summary of an individual's particular experiences relevant to the topic. In addition to presenting astateoftheart summary on the assigned topic, speakers will be asked to outline major problems or challenges relevant to the topic, including infrastructure and administrative matters, and to propose ways of solving some of those problems and encouraging progress in their particular subject areas. Speakers will also be asked to provide the panel with a brief summary of important points they plan to make.

In addition to making a general plan for the panel's next two meetings, panel members began to identify problems to address as they assess theNIH investment in gene therapy research. One issue that the pane will consider, which is not unique to gene therapy research, is how different NIH institutes and centers divide resources between intramural and extramural programs. On average, the intramural program budge is about 11 percent of the overall NIH budget, but there is considerable variation across specific programs and projects. Historically, the first few gene therapy clinical protocols were undertaken by researchers in the intramural program, and there is continued strong interest in pursuing such developments. Is that an appropriate strategy?

This issue is related to a more general question of how institutes and centers coordinate overlapping programs in gene therapy research both across extramural portfolios and in the intramural program. In practical terms, a question for the panel may be framed as follows: Should several institutes and centers focus on a few seemingly tractable genetic disorders, such as cystic fibrosis andGaucher's disease, simultaneously supporting relatively comparable research approaches? Or should early efforts be directed more broadly and targeted for a much more diverse set of diseases?

Other issues that the panel may consider include the following:

- Should there be a special new study section to deal exclusively with gene therapy research and related scientific issues?
- Should NIH efforts to support gene therapy be scaled back rather than accelerated?
- Are recent RFAs issued for specialized gene vector laboratories and for gene therapy programs for specific disorders appropriate at this time? What other diseases or technologies would be appropriate subjects for RFAs?
- What should be done about closing the information gap between the biomedical research community and the wider group of medical practitioners as well as the general public regarding gene therapy?

Future Meeting

The second meeting of the Panel to Assess theNIH Investment in Research on Gene Therapy is scheduled for July 13-14, 1995, atNIH, and the third meeting is scheduled for August 17-18, 1995, in San Francisco, California.

List of Speakers

Duane F. Alexander, M.D. **John I. Gallin, M.D.** Director Director National Institute of Child Health and Warren Grant Magnuson Clinical Center Human Development **Wendy Baldwin, Ph.D.** **Robert A. Goldstein, M.D., Ph.D.** Deputy Director for Extramural Research Director Office of the Director Division of Allergy, Immunology, and Transplantation National Institute of Allergy and Infectious Diseases **James F. Battey, Jr., M.D.** **Michael Gottesman, M.D.** Director, Division of Intramural Research Deputy Director for Intramural Research National Institute on Deafness and Office of the Director Other Communication Disorders **Henning Birkedal-Hansen, D.D.S., Ph.D.** **Richard J. Hodes, M.D.** Director, Division of Intramural Research Director National Institute of Dental Research National Institute on Aging **Francis S. Collins, M.D., Ph.D.** **Claude Lenfant, M.D.** Director Director National Center for Human Genome National Heart, Lung, and Blood Research Institute **Karl Csaky, M.D.** **Michael Lockshin, M.D.** Medical Officer Acting Director National Eye Institute National Institute of Arthritis and Musculoskeletal Diseases **Carl Dieffenbach, Ph.D.** **Harry L. Malech, M.D.** Acting Associate Director Deputy Chief Basic Science Program Laboratory of Host Defenses Division of AIDS National Institute of Allergy and National Institute o Allergy and Infectious Diseases Infectious Diseases **Judith Fradkin, M.D.** **Daniel Rotrosen, M.D.** Chief Chief, Host Defense & Inflammation Endocrine and Metabolic Diseases Division of Allergy Immunology & Program Branch Transplantation National Institute of Diabetes and National Institute of Allergy and Digestive and Kidney Diseases Infectious Diseases **Maria Freire, Ph.D.** **Giovanna Spinella, M.D.** Director, Office of Technology Transfer Health Scientist Administrator Office of the Director Developmental Neurology Branch Division of Convulsive, Developmental and Neuromuscular Disorders National Institute of Neurological Disorders and Stroke **Judith L. Vaitukaitis, M.D.** **Harold Varmus, M.D.** Director Director National Center for Research Resources National Institutes of Health **Robert E. Wittes, M.D.** **Nelson A. Wivel, M.D.** Acting Director Director Division of Cancer Treatment Office of Recombinant DNA Activities National Cancer Institute

REPORT OF THE SECOND MEETING, JULY 13-14, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as co-chairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its second meeting on July 13-14, 1995, at the National Institutes of Health (NIH), Building 31, 9000 Rockville Pike, Bethesda, Maryland 20892. During the course of the two-day meeting, panel members heard from representatives from the academic community and the biotechnology industry who are developing gene vectors and working on clinical protocols in the field of gene therapy. In addition, the committee heard a presentation outlining the impact of patenting on this field. The members of the committee also met for several hours in a closed session.

Vectors: Technical Issues

Initially, researchers have concentrated on developing viruses to serve as vectors for experimental gene transfer and potential gene therapy procedures. Several types of viruses are being studied for this purpose with most efforts focusing almost exclusively on retroviruses. Several other types of virus, including adenovirus (AV), adeno-associated virus (AAV), herpesvirus, and human immunodeficiency virus (HIV), are currently also being developed or at least considered for this purpose. In addition, some research groups are studying non-viral vectors, such as liposomes, cationic detergents, and other chemical ligands, for complexing and carrying DNA molecules into target cells.

Several experts believe that, eventually, these two separate vector strategies may converge as researcher try to develop synthetic or semi-synthetic vectors that incorporate the useful features of viruses and chemical agents. Meanwhile, although specific strategies to build useful vectors have strong advocates, no particular vector has emerged as a clear front runner. Each approach has its own problems, and most of

them also share problems.

For example, except for AAV, these virus vectors integrate randomly, if at all, in the host cell's chromosomes. Moreover, transduction efficiencies for the virus vectors vary widely--in part reflecting their poor ability to integrate into the chromosomes of resting cells. This problem may even affect HIV, despite a widely held notion that it can infect resting cells. Nonetheless, according to Dr. Richard Mulligan, some of the more recently refined retroviral vectors efficiently transduce non-resting target cells, particularly if they carry appropriate LTR sequences and selectable marker genes or, in some cases, specific promoter-enhancer sequences.

Another general problem is that very little research has been done to incorporate externally controllable gene sequences into viral vectors. For instance, regulated beta-globin gene expression is perhaps the most widely studied prototype. However, when this gene is transduced successfully into human cells growing in tissue culture, its expression cannot yet be properly regulated. Some of these difficulties in attaining gene regulation may arise because of the randomness of integration.

In part because gene regulation questions are unanswered, determining the appropriate dosage levels for viral vectors presents another major challenge. For example, according to Dr. Alan Smith, in clinical trials involving patients with cystic fibrosis (CF), there is a concern that the vector and the CFTR gene product it carries may pose problems if they are delivered in too high doses. Because CFTR is ordinarily effective in cells when present at very low levels, low doses of the transferred gene may be required for effectiveness and may be less likely to induce host inflammatory responses.

These considerations raise a more general and potentially serious problem, namely that viral vectors may carry genes--either their own or the particular recombinant genes they are modified to carry--that elicit host immune system responses. This phenomenon might interfere with the efficacy of gene therapy procedures, possibly curtailing long-term expression of transferred genes and prohibiting repeat administration of the therapeutic agent. Other factors, such as counter selection of the transduced cell by immune or other mechanisms and the randomness of integration, may also contribute to apparent low transduction efficiencies and/or short-lived expression of transferred genes.

Dr. Smith said that cationic lipid vectors are being improved and now perform as much as 500-fold more effectively than naked DNA but are still less effective than is the AV vector in rodent model systems. A potential advantage of cationic lipids is that they can be administered repeatedly to rodents. However, at high doses they induce some focal inflammatory responses, albeit without evidence of eliciting antibodies or provoking T cell activation. Dr. Smith speculated that cationic lipids activate macrophage cells.

Additional advantages and problems associated with specific vector candidates:

- **Retroviral Vectors** Although retroviral genes have been extensively modified to ensure that these vectors cannot replicate and are unlikely to recombine, this extensive modification makes them that more difficult to produce. For example, sometimes several packaging cell lines are needed to produce the vectors, and these cell lines are difficult to derive and maintain. Integration of retroviral vectors into the host chromosome is random, and expression levels of the transgene vary and often are unacceptably low.

  In addition, host cell range tends to be narrow, although introduction of genes from other viruses such as vesicular stomatitis virus (VSV) may help in broadening that range. However, the presence of VSV genes may introduce new toxicity problems, leading to damage or killing of the host cell.

- **Adenovirus (AV)** Several research groups are investigating whether systematic removal or modification of AV genes can reduce host flammatory responses when this virus serves as a gene vector.

  Dr. James Wilson said that other approaches to controlling the inflammatory response are being considered, including production of antibodies to block T cell activation, use of agents such as the drug cytoxan to block T cell proliferation, and use of cytokines to reduce or block production of neutralizing antibodies.

  Dr. Thomas Shenk said that several AV genes influence tumor formation in animal model systems and malignant transformation of cultured cells. Thus, AV represents a potential problem when modified versions of the virus are used as vectors, even though AV has not been observed to cause human tumors. He also is studying the molecular and cellular events required for AV to recognize, bind to, and penetrate target cells, and to deliver and integrate the genes it carries to the target cell nucleus.
- **Adeno-Associated Virus (AAV)** AAV, when modified to serve as a vector, lacks certain control sequences and has limited DNA (4.4 kb) carrying capacity, according to Dr. Kenneth Berns. Moreover, he pointed out that the virus is difficult to produce in high titers and needs to be purified in cesium chloride gradients, a laborious procedure. Because AAV integration is site specific, at least in the wild type, there is a question whether repeat dosing with this vector will be possible because follow-up doses may be routinely excluded from the AAV-occupied site on the host chromosome. In some researchers' hands, AAV has a very low transduction efficiency unless AV or AV genes are also present.

Clinical and Animal Model Studies: Technical Issues

Invited speakers described gene therapy clinical trials involving a range of diseases, including inherited conditions such as adenosine deaminase (ADA) deficiency and cystic fibrosis (CF), a range of malignancies, and AIDS. Some of the justification for conducting clinical trials at this relatively early stage of gene therapy's development is that other well-tried approaches have not yielded satisfactory therapies for treating these usually deadly diseases. Another problem, cited frequently in the case of CF and applicable to several other cases, is that animal model systems are far from perfect, sometimes making results from gene transfer experiments incomplete or misleading.

Yet another set of problems entails uncertainties over the target cells for gene transfer procedures. Dr. Arthur Nienhuis noted that several issues may help to account for low overall gene transfer efficiency in clinical settings. These include the phase of the cell growth cycle that a particular target stem cell may be in, the current unavailability of effective cytokines to regulate that cycle, difficulties in stimulating specific viral receptor production by the cell, and problems in improving the transduction efficiency of target cells. Stimulation with cytokines or, alternatively, the introduction of drug resistance markers and subsequent use of the corresponding drug may provide ways of expanding specific transduced target cell populations. However, Dr. Nienhuis cautioned that such approaches are still at a very early, preclinical stage of development.

Results from clinical trials so far are limited. Relatively few patients have been treated; measures of biological response are often not adequately sensitive, except in cases where host inflammatory responses have been reported; the effects observed seem to be erratic; and the reporting of effects so far has been almost entirely anecdotal, rather than in peer reviewed publications.

According to Dr. Ronald Crystal, AV-delivered CFTR genes may be expressed along airways of CF patients as many as four days after being administered; however, that expression is observed in only a low percentage of the patients treated. According to Dr. James Wilson, in other experiments involving CF patients, expression of the CFTR gene is rare, not stable, but also not toxic. Although sustained expression is attained in knock-out mice, efforts to introduce theCFTR gene in other animal model systems tend to induce immune responses directed to vector (AV) genes.

Clinical results are also variable in the few ADA patients who are partaking in gene transfer experiments, according to Dr. Michael Blaese. One youngster has been infused 11 times over 23 months with her own T cells after they were treated with a retrovirus carrying an ADA gene, and ADA+ T cells have persisted for two years following the eleventh infusion. He said there is one copy of vector per peripheral T cell, and a positive signal for circulating mRNA (earlier, that signal was "intermittent"). A complicating factor is that PEG-ADA is still being administered to the patient, albeit in a low dose that was established before she more than doubled in weight.

The results for a second child under the same treatment regime are more ambiguous but apparently less promising. However, Dr. Blaese said that three other children whose cord blood was treated at birth show persistent expression of the vector after more than 12 months following the procedure. In addition, good expression of the ADA retroviral-delivered gene was obtained in vitro from foreskin cells obtained from two of these patients, suggesting that small skin grafts using modified cells might be an effective alternative means of delivering the corrective ADA (or other) genes.

Results from gene transfer experiments involving AIDS or cancer patients are scanty. For example, in some cases the HIV+ member of an identical twin pair develops positive skin responses following a gene transfer procedure, but whether this change will lead to clinical benefits is not yet known.

Dr. Philip Greenberg also refers to "transient" antiviral effects and "proof of concept" in gene transfer experiments involving modified HIV genes in patients with AIDS.

A wide range of clinical experiments involving patients with a variety of cancers is under way. Dr. Blaese said there is some evidence of efficacy, such as tumor shrinkage in patients withglioblastomas. Some of the protocols call for the gene transfer procedure to induce immune system responses against the tumor, according to Dr. Gary Nabel. In some cases, patients appear to go into long-term remission; in other cases the effects are transient. Partial effects are commonplace in cancer treatment, and gene therapy approaches therefore may find acceptance as a useful addition to the therapeutic arsenal.

Dr. Nabel and Dr. John Mendelsohn pointed out that, in gene transfer experiments involving cancer patients, better measures of biological activity are needed. This need is particularly acute in early tests involving patients with advanced disease when other treatments and other clinical abnormalities make assessment of a single experimental procedure exceedingly difficult.

Responses to the question of whether the field is ready for clinical trials

- Dr. Mulligan: Too much of current research is "not worth taking to patients." The field needs "wise people to prune and avoid copy cat" projects.

- Dr. Smith: "We don't know it won't work." Regarding uncertainties about identifying and successfully targeting epithelial stem cells in human airways, he said that treatments would need to be repeated because cells are expected to turn over every 60-80 days. Also, problems have been seen in animal models where the transgene was expressed in excess; transfection is inconsistent in monkeys when high-dose vectors are tested but successful at low doses in cotton rats; and the goal is not specifically to achieve stem cell integration or to "duplicate" nature but to produce a "useful" therapeutic agent.
- Dr. Crystal: Through clinical trials, investigators are "learning how to evaluate" the gene transfer procedures. In the case of trials involving CF patients, currently antibody-based tests are not sensitive enough to detect the product of thetransfected CFTR gene; there are other difficulties with PCR-based assays. Non-human primates, such as rhesus monkeys, are not a reliable model for CF.
- Dr. Mendelsohn: Oncologists have taken drug studies as far as seems possible so the "new approach of gene transfer is exciting ... and needs to be backed."
- Dr. Shenk: If gene transfer procedures appear to work in animal models of some diseases, particularly cancer, they are probably ready for clinical trials. For other diseases, such as CF, particular problems with vectors and gene delivery came to light only because of findings from early-stage clinical trials. Sometimes researchers are unaware of a phenomenon until they do clinical trials and would not have known to look for it during animal experiments. Once appreciated, the phenomenon may better be studied in model systems. However, a moratorium on clinical trials is not warranted.

Basic and Clinical Infrastructure and Training Issues

Speakers identified several areas of basic biology research that need greater emphasis:

- better understanding of hematopoietic cells and of bone marrow transplantation; stem cell heterogeneity; lung epithelial biology; inflammatory responses; and apoptosis, which may prove important for treating diseases such as cancer and AIDS;
- better understanding of basic virology and manipulations needed to improve vectors and their delivery to appropriate cells in target tissues and organs or to tumors; and
- better models for preclinical studies of disorders that may be subject to gene therapy approaches; however non-human primate models cannot replace clinical research because they are difficult to develop and costly to use.

Speakers also identified several logistical and pragmatic barriers to overcome to foster progress in gene therapy research:

- Means are needed for producing high amounts of vectors of suitable quality for use in small-scale clinical experiments; there is disagreement whether NIH should sponsor GMP vector production facilities.
- More sensitive and reliable assays are needed for assessing the biological activity of transferred genes and clinical end points.
- Novel relations among government, industry, and academic institutions will be needed at the

research level and as novel, clinically useful reagents are developed; more than 50 companies are said to be doing gene therapy-related research.

- Industry representatives referred to regulatory impediments and criticized the current clinical protocol review process involving oversight by the NIH RAC and FDA.
- Some participants raised the issue of conflicts of interest.
- One speaker suggested that more international collaborations should be encouraged.

Several speakers referred to training needs, but there is not full agreement on the kind of training that should be emphasized. In general, participants said they prefer rigorous training in basic scientific disciplines, even for young clinical investigators who want to work in the field of gene therapy. There is some sense that, if gene therapy develops rapidly into a successful clinical modality, new means will be needed to integrate these approaches into the current system for delivering health care, which itself is rapidly changing.

Patent Issues

Because many patent applications pertaining to gene therapy technology are still pending, their impact on this emerging field remains difficult to predict, according to Ms. Rebecca Eisenberg. She recommends that research institutions rely more on non-exclusive licensing agreements as a way of circumventing several potential problems and thereby not hindering the efficient development of this field.

The U.S. Patent and Trademark Office (PTO) has issued several broad-based patents covering fundamental gene therapy technologies, including a patent granted to NIH and licensed exclusively to Gene Therapy, Inc., covering ex vivo gene therapy and another patent granted to the University of Michigan and licensed exclusively to Genovo that covers any viral gene therapy vector carrying theCFTR gene, which is impaired in individuals with CF.

Ms. Eisenberg said that these examples as well as other signs indicate this field of biotechnology is likely to be "more littered" with patents than is the earlier emerging field of biotechnology involving the discovery and development of therapeutic proteins.

Ms. Eisenberg attributes this difference to the fact that universities and other research institutions are being even more aggressive now than a few years ago in pursuing patent protection for intellectual property their researchers are developing. The Bayh-Dole Act, which specifies that such institutions may retain ownership in patents arising from federally sponsored research, now provides strong incentives for pursuing patents--raising expectations in the university community that royalties from licensing agreements eventually will become a significant source of revenue.

Although in some noteworthy cases involving biotechnology inventions universities are benefitting from significant royalty payments, there are potential problems to face from the flurry of patent applications being put forth in the field of gene therapy, according to Ms. Eisenberg. Perhaps chief among them is that research teams and clinicians may, in effect, be faced with a series of "toll booths" along the road to developing and implementing effective gene therapy procedures. She says that research groups may be hemmed in and financially pinched if they have to enter into complex cross-licensing agreements or if institutions set royalty requirements at levels that are too high. Additional complications include potential priority disputes between competing "inventors," disagreements over ownership when researchers at

several institutions are collaborating on a project, and differences arising because some researchers such as medical geneticists tend not to patent their work, whereas other researchers such as molecular biologists do so.

Future Meeting

The third meeting of the Panel to Assess the NIH Investment in Research on Gene Therapy is scheduled for August 17-18, 1995, in San Francisco, California.

List of Speakers

Kenneth Berns, M.D. Gary J. Nabel, M.D. Deparment of Microbiology Howard Hughes Medical Institute Cornell University Medical College University of Michigan Medical Center New York, NY 10021 Ann Arbor, MI 48109-0650 **Michael R. Blaese, M.D. Arthur W. Nienhuis, M.D.** National Center for Human Genome Research St. Jude Children's Research Hospital National Institutes of Health Memphis, TN 38101 Bethesda, MD 20892 **Ronald G. Crystal, M.D. Thomas Shenk, Ph.D.** New York Hospital-Cornell Medical College Department of Molecular Biology New York, NY 10021 Princeton University Princeton, NJ 08544 **Rebecca Eisenberg, J.D. Alan E. Smith, Ph.D.** University of Michigan Law School Genzyme Corporation Ann Arbor, MI 48109-1215 Framington, MA 01701-9322 **Philip D. Greenberg, M.D. James M. Wilson, M.D.** University of Washington University of Pennsylvania Seattle, WA 98195 Philadelphia, PA 19104 **John Mendelsohn, M.D.** Department of Medicine Memorial Sloan-Kettering Cancer Center New York, NY 10021-6094 **Richard Mulligan, Ph.D.** Somatix Therapy Corporation Alameda, CA 94501 and Massachusetts Institute of Technology Whitehead Institute Cambridge, MA 02124

REPORT OF THE THIRD MEETING, AUGUST 17-18, 1995

With Dr. Stuart H. Orkin and Dr. Arno G. Motulsky serving as co-chairs, the Panel to Assess the NIH Investment in Research on Gene Therapy convened for its third meeting on August 17-18, 1995, at the Sir Francis Drake Hotel, San Francisco, California. During the first day of the two-day meeting, panel members heard from several representatives of the academic community and the biotechnology industry who are developing gene vectors and working on clinical protocols in the field of gene therapy. The panel members also heard from researchers outside this field who are working at a more basic level. Some of these researchers are skeptical about certain developments in gene therapy, calling some of them misguided, others premature. On the second day, the members of the committee met in a closed session to outline the report they plan to deliver to NIH Director Harold Varmus.

The Case for Re-Emphasizing Basic Research

Several investigators who appeared before the panel made a case for re-emphasizing basic research and pursuing other strategies for treating some of the diseases that researchers in the field of gene therapy have been studying. One line of argument is that alternative biochemical manipulations appear simpler to apply than gene transfer techniques and might reach fruition sooner. Another line of argument is that gene transfer approaches are premature because not enough is understood in the field of stem cell biology, a vital prerequisite for success in gene therapy.

Some of these investigators criticized current proponents of gene therapy for portraying the field in unrealistic terms and misrepresenting progress as more rapid than it has been. For example, Dr. Joseph Goldstein called for greater realism in the way these researchers present views of their field to the public.

He also pointed out that the development of any new therapeutic product is a laborious, time-consuming effort.

Dr. Goldstein said that some of the diseases now targeted by gene therapy researchers might be treated sooner, by other strategies, if investigators pursued more traditional studies into thepathophysiologic basis of the diseases in question. He cited several examples where this alternative approach has paid off either recently or several decades ago. For instance, prednisone treatment reverses steps in a defective sterol metabolic pathway that otherwise leads to masculinization. In a more recent development, an inhibitor of cholesterol production (lovastatin) overcomes a LDL receptor deficiency and, by lowering cholesterol levels, helps to prevent coronary heart disease.

Dr. Goldstein also referred to several genetic diseases that arise because of protein trafficking abnormalities. In some of those cases, the critical mutations lie outside the functional coding region of the enzyme product and, instead, serve to misdirect nascent proteins, which are transported into the wrong biological compartments. He called for basic research that could provide an alternative means to gene therapy for correcting such defects.

Dr. Irving Weissman and Dr. Goldstein said that studies with animal models deserve greater emphasis than they are receiving by researchers who are moving quickly from basic research to the clinic to test new ideas about gene therapy. This general problem is particularly applicable to several unsolved problems involving stem cells, which are important but elusive targets of many gene transfer protocols in which long-term gene expression is a major goal.

Dr. Weissman pointed out that stem cell biology in humans and mice is essentially equivalent. From studies on mice, investigators have learned that there are three critical subsets of stem cells in bone marrow and that the most desirable subset for gene transfer is the rarest and is very difficult to work with.

A key problem in the use of retroviral vectors is to determine which factors will induce self-renewing stem cells to divide. Without such detailed information that can be applied practically, gene transfer procedures will likely fail because genes will not be integrating into target progenitor cells. Dr. Weissman said that, with such fundamental obstacles to human gene transfers, it may make sense to focus instead on activating genes that are already present rather than on replacing defective or missing genes.

Dr. Victor Dzau pointed out that, for certain clinical conditions including several that affect the cardiovascular system, short-term rather than long-term gene expression may be all that is needed to address specific problems. Moreover, in a rabbit model system, studies indicate that localized high pressure can improve DNA transduction rates, enabling antisense oligonucleotides to block transiently a cell-proliferative response that otherwise may interfere with surgically grafted blood vessels. Experiments indicate that high pressure also enhances the delivery of oligonucleotides into cultured human cells, improving the efficiency of transduction.

Dr. Gerald Crabtree described the use of synthetic, lipid-soluble dimerizing reagents that can be used to bring cellular regulatory proteins into covalent juxtaposition, thereby changing their functional status. For example, with appropriate dimeric reagents, specific transcriptional factors might be modified in such a way that they permanently activate this process, meaning that a transgenic cell produces high levels of the designated gene product. Another potential use of such dimerizing reagents would be to cross-link specific cell receptors to induce apoptosis. Although this approach shows promise and many other applications are imaginable, studies are limited so far to cellular systems and considerable work will be needed before animal model studies can be undertaken.

The Case for Simultaneous Basic and Clinical Research

Several investigators who came before the panel said that the rapid movement from the laboratory to the clinic to test gene transfer protocols sometimes is essential. Dr. W. French Anderson said that, with more than 120 clinical protocols now approved, the nearly five-year-old field of gene therapy research is showing healthy progress. He also predicted that it will be 15 to 20 years before the full potential of current research will be realized.

Dr. Flossie Wong-Staal pointed out that in vitro studies or animal models of AIDS are far from adequate, making it best to go forward rapidly with small, focused clinical trials to test gene transfer procedures. Although the rationale for using ribozyme genes to block HIV gene expression appears sound when tested at the cellular level, many questions, such as the extent to which target cells in patients will be genetically modified and then selected and whether HIV will develop resistance to the ribozyme, can only be addressed through clinical studies.

Dr. Anderson outlined a variety of gene therapy research studies at his institution, suggesting that this locally concentrated diversity of interests and ideas is another sign that this field is healthy and populated with creative young investigators. He also described a long-term project that involves making a series of improvements in a current retroviral-based vector that could extend its half-life in the host circulatory system, increase its efficiency of binding to and entering specific target cells of the host, improve its chances of delivering genes for long-term expression, and eventually lead to a readily injectable gene-delivery product. Efforts to realize these goals are only at the "very beginning."

Other current basic research developments may eventually help solve some of the challenges that investigators conducting human gene transfer protocols now face. For example, Dr. Donald Kohn described efforts to modify the long terminal repeat (LTR) in a retroviral vector now being used in gene transfer protocols as a way of extending the expression of transferred genes after they are delivered to target cells. Hematopoietic cells from mice are providing a valuable model in which to study this problem, and some results indicate that methylation within the LTR correlates with the disappearance of transferred gene expression.

In a model system in which human bone marrow cells are introduced into immunologically deficient nude mice, Dr. Kohn and his collaborators find that the addition of stroma enhances gene transfer in vitro and also extends long-term expression of the transduced genes. The impact of growth factors on these steps is also being evaluated. Dr. Kohn said that, despite the value of this information from experiments in mice, clinical trials are needed to understand in detail how each of these steps work in humans.

One important problem that has come to light from early gene transfer clinical studies is that host immune responses may abbreviate expression of transferred genes. Dr. Paul Tolstoshev described efforts to develop sophisticated vectors that can overcome this problem. Less immunogenic vectors are being constructed for use in conjunction with immunosuppressive agents such as dexamethasone or cyclosporin that can reduce immune system responses, including deleterious inflammatory reactions.

Academic, Industry Representatives' Comments on Policy Questions

Industry and academic representatives said that clinical trials are an important element of gene therapy, providing data that have helped in choosing among models and in other ways are proving essential for the development of this field. Dr. Wong-Staal said that the cost as well as the complexity of current regulatory requirements impose barriers on efforts to design and conduct small-scale clinical trials. Moreover,

simplifying annual reporting requirements would be helpful to investigators.

Dr. Anderson pointed out that progress is more likely to be rapid if individual investigators--rather than a central committee--direct research decision making. He also recommended that the development and use of vectors made in NIH-supported specialized laboratories not be restricted to only those researchers whose work is being supported by NIH. He was less certain whether a policy of limiting such vector development to research on orphan diseases should be adopted.

Dr. Barrie Carter pointed out that efforts to begin the first clinical trials and subsequent efforts to test additional gene transfer protocols in clinical settings are driving a great deal of basic research in biology. Although NIH programs provided the fundamental research from which gene therapy derives, industry now furnishes enormous resources to further these developments. He noted that NIH spends about $200 million annually on gene therapy research, and this amount represents less than 2 percent of total NIH research expenditures. He recommended that NIH spending be maintained at this level, concentrating in several program areas such as gene delivery systems, target cell biology, and preclinical models.

Dr. Carter noted that basic and clinical research within the NIH Intramural Program is a valuable component of overall efforts in the field of gene therapy. He also praised the role NIH plays in supporting programs in basic research on viral vectors and at General Clinical Research Centers. However, he questioned the value of NIH setting up new gene vector production facilities, suggesting that industry can do a better job producing vectors. Dr. Tolstoshev noted that companies are conducting a great deal of fundamental research on gene vectors and, in many cases, these vectors are being made available to university researchers for testing and evaluation.

Industry representatives pointed to several technology transfer arrangements that are helpful to them, despite specific obstacles which sometimes arise. For example, Cooperative Research and Development Agreements (CRADAs) are now being used extensively to establish relationships between companies and NIH investigators in the field of gene therapy. Dr. Tolstoshev said that the many CRADAs established between his company, Genetic Therapy, Inc. (GTI), and individual NIH investigators are particularly helpful in leveraging the company's expertise. Other types of agreements, including material transfer agreements and scientific collaborations between industry and university researchers, are providing a major source of funding for this developing field, and that source could grow larger as major established pharmaceutical companies take a greater interest in gene therapy.

Legal and policy difficulties sometimes have made CRADA negotiations drawn out and cumbersome. Dr. Tolstoshev noted that, by eliminating a clause calling for "reasonable pricing" of drugs and other products that may flow from a CRADA, NIH removed what had become an important stumbling block for industry. However, he also said that protracted negotiation of the legal terms of many CRADAs can still be an impediment to efficient technology transfer.

Industry representatives pointed to other important policy issues, including a need for clear-cut patenting policies and the relative value of exclusive versus non-exclusive licensing agreements. Industry representatives said that, in general, licensing agreements granting particular companies the exclusive right to commercialize intellectual property developed by NIH investigators are more likely to provide essential incentives to pursue development than are non-exclusive agreements.

List of Speakers

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